Turkish Journal of Fisheries and Aquatic Sciences 9: 77-83 (2009)

Ontogeny of Biochemical Phases of Fertilized Eggs and Yolk Sac Larvae of Gilthead Seabream (*Sparus aurata* L.)

Mehmet Naz[1,*]
[1]Mustafa Kemal University, Faculty of Fisheries Sciences, Hatay, 31040, Turkey.

* Corresponding Author: Tel.: +90.326 6141693; Fax: +90.326 6141877;
E-mail: mnaz@mku.edu.tr

Received 21 July 2008
Accepted 05 January 2009

Abstract

Biochemical compositions of fertilized gilthead seabream (*Sparus aurata*) eggs and yolk sac larvae were determined at different periods (after hatching, 0th, 24th, 48th and 96th hours). Results showed that the yolk sac larvae and eggs contain more monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids than saturated fatty acids (SFAs). The changes observed in essential (EAA) and non-essential amino acid (NEAA) contents of fertilized eggs and yolk sac larvae through the experimental period were significant ($P<0.05$). Amylase and tyripsin activity was detected in fertilized eggs. Leucine alanine (LEU-ALA) peptidase activity was higher than other enzymatic activities throughout the experimental period. Aminopeptidase N(LAP) activity decreased from fertilized egg to hatching. After hatching, LAP activity increased until 24th hour and then decreased up to the beginning of exogenous feeding ($P<0.05$).The lowest alkaline phosphatase activity (AP) was taken from fertilized eggs. Then, AP activity tended to increase until the end of experimental period ($P<0.05$). In conclusion, our results can give information about the nutritional requirements of seabream larvae at the start of exogenous feeding.

Keywords: gilthead seabream, *Sparus aurata*, egg, yolk-sac larvae, digestive enzyme, amino acid, fatty acid.

Introduction

The gilthead seabream (*Sparus aurata*) is an important species in the Mediterranean Sea due to its high economic value and its adaptation ability to different environmental conditions such as salinity and temperature. Due to its importance in the Mediterranean aquaculture, postlarval stages of seabream have been comprehensively studied under culture conditions (Kolkovski *et al*., 1993; Yufera *et al*., 1993; 1995; 1996; 1999; Parra and Yufera, 2000). However, literature about the biochemical compositions and also digestive characteristics of its early life history is scarce.

It is well known that yolk sac stage represents an important developmental period for all fish larvae. At this stage, the significant changes in the larval body take place before exogenous feeding. Also, the energy in the yolk is used for growth, development and activity, additionally; both protein and lipid are major energy fuels during the embryonic and yolk sac stages of fish larvae (Fyhn, 1993; Polat *et al*., 1995; Sargent, 1995; Verreth *et al*., 1995). However, most information on the protein and lipid metabolism in fish is derived from studies on juvenile and adult specimens. These studies show that marine fish larvae contain eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), which are considered as essential, whereas as freshwater fish are capable to synthesize these important highly unsaturated fatty acids (HUFAs) from their C18 precursors, e.g. linoleic acid(18:2n-6) and linolenic acid (18:3n-3) (Henderson and Tocher, 1987; Sargent *et al*., 1989).

On the other hand, amino acids (AAs) are the major substrates of aerobic metabolism during the development of embryo and yolk sac larvae of marine fish species which have pelagic eggs (Fyhn, 1989; Ronnestad *et al*., 1992; 1994; 1999; Finn *et al*., 1995; Seoka *et al*., 1997; Sivaloganathan *et al*., 1998). The profile of essential amino acids (EAAs) of a fish body is generally considered to be a good indicator of their AA requirements (Cahu and Zambonino Infante, 2001). However, little is known about the AA requirements of larval fish and their possible ontogenetic changes.

The digestion mechanisms in marine fish larvae have been particularly studied during the last two decades as a means of understanding the nutritional requirements of animals and the effect of dietary constituents on digestive enzyme activity (Zambonino Infante and Cahu, 2001). Despite many studies on the larval digestive system, the understanding of the ontogeny of digestive function in larval fish is still incomplete.

The knowledge of the biochemical compositions and the digestive enzyme activities of fertilized seabream (*Sparus aurata*) eggs and yolk sac larvae provide better understanding on the nutritional requirements at the start of exogenous feeding. Consequently, the understanding of the nutritional dynamics could be provided an important contribution for the enhancement of larval and juvenile quality. The aim of this study was to determine the changes in nutrient compositions as well as the digestive enzyme activities during the egg and yolk sac stages of gilthead seabream (*Sparus aurata*).

Materials and Methods

Broodstock, Egg Incubation and Larval Rearing

This study was carried out at the Mediterranean Fisheries Research, Production and Training Institute. Ten females (2.1 kg mean weight) and ten males (1 kg mean weight) of gilthead seabream (*Sparus aurata*) broodstock were selected from wild breeders and stocked in a 10 m^3 tank with a seawater supply of 30 l/min. Throughout the experimental period, the water temperature was 16±0.5ºC. Spawned eggs were immediately collected into a recuparator and then buoyant viable eggs were separated from sinking dead eggs. Fertilized eggs were disinfected with iodine solution (50 mg L^{-1}) and incubated in 150 L incubators supplied with a gentle flow of sea water of 16±0.5ºC. Newly-hatched larvae were transferred from incubators to fiber glass tanks (300 L) with black walls. Water temperature was controlled by pipe heating systems and automatic transformer equipment was calibrated at ±0.5ºC. The rearing tanks were held in complete darkness and stocked in triplicate with newly hatched larvae (approximately 100 larvae L^{-1}).

The water flow rate was adjusted to exchange 10% of the total volume of the tanks every hour. The tanks were supplied with running sea water which had been filtered through a UV filter. Salinity was 36.5±1.5 ppt throughout experimental period. The oxygen levels were maintained above 6.5 mg L^{-1} with liquid oxygen systems. Air and fresh sea water were introduced into the bottoms of the tanks to prevent water stratification.

Sampling and Analytical Methods

Before the eggs were placed in the incubators, the samples for biochemical analyses were taken. After hatching, sampling was made at 0^{th}, 24^{th}, 48^{th} and 96^{th} hours. The samples were immediately stored at -196 ºC until assayed.

Whole body larvae and fertilized eggs were homogenized in 5 volumes (v/w) of ice cold distilled water. Trypsin and amylase activities were assayed according to Tseng *et al.* (1982) and Metais and Bieth (1968), respectively. Alkaline phosphatase(AP), leucine aminopeptidase N(LAP) and leucine alanine peptidase(LEU-ALA) were assayed according to Bessey *et al.* (1946), Maroux *et al.* (1973) and Nicholson and Kim (1975), respectively. Enzyme activities were expressed as µmoles of substrate hydrolyzed per minute per mg protein (i.e., U/mg protein) at 37ºC for AP, LAP and at 25ºC for trypsin. Amylase activity was expressed as the equivalent enzyme activity which was required to hydrolyse one mg of starch in 30 min at 37ºC. LEU-ALA activity was expressed as nmoles of substrate hydrolyzed per minute per mg protein (i.e., U/mg protein) at 37ºC.

Fertilized eggs and yolk sac larvae were taken and esterified for fatty acids (FAs) analysis according to Garces and Mancha (1993). Analysis of FA methylesters were carried out on GC-MS equipped with a SP-2330 fused capillary column (30x 0.25 mm) using hydrogen as carrier gas and a temperature gradient programmed from 120°C to 220 °C (5°C/min). The temperature of the injector and of the detector was 240°C and 250°C, respectively. Methylesters were identified by comparison with a known standard mixture of FAs.

AA contents of fertilized eggs and yolk sac larvae were analyzed according to AOAC (1995). AA contents of samples hydrolyzed were determined using GC Varian 3800 equipped with a ZB-AAA (10 m x 0.25 mm) column. (The Scientific and Technical Research Council of Turkey) the using helium as carrier gas and a temperature gradient programmed from 110°C to 310°C (30°C/0.3 min). The temperature of the injector and of the detector was 250°C and 320°C, respectively. The concentrations of soluble protein in fertilized eggs and yolk sac larvae were determined by the Bradford (1976) method using bovine serum albumin as a standard. The experiment was terminated at the beginning of exogenous feeding.

Statistical Analysis

Results are expressed as means ± standard deviation. Statistical comparisons were conducted using SPSS 9.0 for Windows (SPSS, 1993). A One-Way Analysis of Variance (ANOVA) was performed to statistical comparisons. Duncan's multiple comparison tests was used to discover differences between means. Differences were considered statistically significant when $P<0.05$.

Results

During the experimental period, the average temperature and salinity in the tanks were 16±0.5ºC and 36.5±1.5 ppt, respectively. These values were not significantly different between experimental tanks ($P>0.05$).

The developmental pattern of the two pancreatic enzymes such as amylase and trypsin are given in Table 1. Amylase activity remained relatively constant from fertilized eggs to hatching ($P>0.05$) and followed by a sharp increase in amylase activity continued until 24^{th} hour ($P<0.05$). After 24^{th} hour, a sharp decrease at 48^{th} hour was observed ($P<0.05$) and then activity value increased at the end of the experimental period ($P<0.05$). Trypsin activity was determined in fertilized eggs and then these activities increased until 48^{th} hour ($P<0.05$). After 48^{th} hour, trypsin activity decreased until 96^{th} hour ($P<0.05$). The changes observed in the activities of LEU-ALA, LAP and AP are given in Table 1. LEU-ALA activity was higher than other enzymatic activities observed in fertilized eggs and newly hatched larvae of seabream.

The highest LEU-ALA activity was found in fertilized eggs (P<0.05). After hatching, LEU-ALA activities decreased until 96th hour (P<0.05). LAP activity declined from fertilized egg to hatching. After hatching, LAP activity increased until 24th hour and followed by a sharp decrease in LAP activity continued until 96th hour (P<0.05). The lowest AP activity was measured at fertilized eggs. AP tended to increase until the end of endogenous feeding. Significant differences in the AP activities were observed throughout the study (P<0.05).

The AA contents in fertilized eggs and yolk sac larvae are given in Table 2. The changes observed in EAA and non-essential amino acid (NEAA) contents of fertilized eggs and yolk sac larvae through the experimental period were significantly different (P<0.05). The amounts of EAA and NEAA at the end of the study decreased to 38.42% and 30.92%, respectively. The predominant AAs observed in *S. aurata* eggs were the leucine, lysine, valine, alanine, proline, aspartic acid, phenylalanine and glutamic acid. EAAs observed at the end of the endogenous feeding were the leucine, isoleucine, methionine, phenylalanine, threonine and valine. The most depleted EAAs until 96th hour were the histidine and lysine (P<0.05). Threonine and phenylalanine remained relatively constant through the experimental period (P>0.05). However, alanine, tyrosine, serine, proline and glutamic acid were determined as the most depleted NEAAs (P<0.05). Aspartic acid amount dropped until hatching and followed by a sharp increase in aspartic acid amount continued until 48th hours. After 48th hour, a sharp decrease in the amount of aspartic acid was observed (P<0.05).

Table 3 shows the FA contents in fertilized eggs and yolk sac larvae. FA results revealed that the yolk sac larvae and eggs contain more monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids than saturated fatty acids (SFAs). In the present study, unsaturated fatty acid content was higher than SFA content. Dominant SFAs were 14:0, 16:0 and 18:0, whereas, dominant MUFAs were 16:1 and 18:1n9c. The highest PUFA was 22:6n-3. In addition, essential fatty acids that affect egg quality in *S. aurata* such as EPA, DHA, arachidonic acid (ARA), MUFA and PUFA were recorded as 2.3255±0.0417,

Table 1. The digestive enzyme activities in fertilized eggs and yolk sac larvae of gilthead sea bream at different periods of development (Mean±SD; n=3)

Digestive Enzymes		prelarval stages after hatching(hours)			
	Egg	0th	24th	48th	96th
Amylase (U/mg protein)	16.682±0.27^{c}	16.389±0.14^{c}	52.429±0.48^{d}	0.752±0.05^{a}	9.597±0.3^{b}
Tyripsin (mU/mg protein)	23.517±0.5^{a}	32.742±0.85^{b}	64.626±0.58^{c}	93.369±0.68^{e}	82.781±0.134^{d}
LAP (mU/mg protein)	799.34±1.53^{c}	708.27±1.01^{b}	809.08±1.91^{e}	800.21±0.9^{d}	695.64±0.33^{a}
AP (mU/mg protein)	72.25±0.92^{a}	130.62±0.62^{b}	224.88±0.2^{c}	312.54±0.62^{d}	325.55±0.6^{e}
LEU-ALA (mU/mg protein)	2849.85±1.72^{e}	2481.61±1.11^{d}	2435.28±1.08^{c}	2280.3±1.19^{b}	1998.66±0.9^{a}
LAP(x1000)/ LEU-ALA	280.48±0.69^{a}	285.408±0.41^{b}	332.235±0.642^{c}	350.926±0.57^{e}	348.054±0.32^{d}

Means with the same superscript are not significantly different at experimental periods (P>0.05).

Table 2. The amino acid contents in fertilized eggs and yolk sac larvae of gilthead sea bream at different periods of development (Mean±SD; n=3)

Amino acids		prelarval stages after hatching(hours)			
(mg/100 g)	Egg	0th	24th	48th	96th
Histidine	101.73±1.96^{a}	149.2±6.05^{c}	129.2±4.75^{b}	nd	nd
Leucine	509.4±8.34^{c}	664.4±3.46^{d}	511.6±1.99^{c}	500.03±3.2^{b}	358.9±0.62^{a}
Isoleucine	288.36±3.81^{b}	369.36±9.32^{d}	334.86±2.31^{c}	288.1±6.6^{b}	191.93±4.48^{a}
Lysine	301.36±5.08^{a}	298.43±2.63^{a}	394.56±6.91^{c}	314.46±2.17^{b}	nd
Methionine	105.23±2.31^{b}	171.43±4.82^{e}	135.53±3.46^{d}	123.3±0.43^{c}	93.16±2.41^{a}
Phenylalanine	251±16.28^{a}	301.4±6.76^{b}	289.46±19.77^{b}	357.26±22.59^{c}	242.56±6.35^{a}
Threonine	196.73±0.92^{a}	304.8±4.5^{d}	260.73±0.49^{c}	240.43±1.3^{b}	193.23±2.98^{a}
Valine	372.7±3.65^{d}	411.76±0.49^{e}	348.5±2.06^{c}	335.66±5.68^{b}	229.63±2.79^{a}
Tyrosine	251.9±2.06^{c}	273.4±0.62^{d}	220.16±0.46^{b}	219.26±3.78^{b}	205±6.035^{a}
Alanine	527.76±6.43^{d}	481.13±3.94^{c}	390.3±3.72^{b}	394.26±1.35^{b}	277.2±0.2^{a}
Glycine	163.83±1.27^{a}	305.5±2.19^{d}	266.7±2.62^{c}	309.76±5.37^{d}	246.86±3.8^{b}
Serine	156.13±0.66^{d}	388.3±5.28^{e}	104.76±1.53^{c}	74.06±2.3^{b}	68.43±3.4^{a}
Proline	382.23±8.91^{e}	309.53±9.9^{d}	217.36±2.54^{b}	232.6±2.19^{c}	138.2±2.7^{a}
Aspartic acid	380.1±4.39^{b}	293.8±1.96^{a}	391.3±1.9^{c}	572.63±3.77^{d}	389.76±7.1^{c}
Glutamic acid	652.4±7.84^{d}	426.66±2^{b}	517.5±1.15^{c}	669.06±4.79^{e}	411.43±7.07^{a}
TFAA	4640.9	5149.133	4512.567	4630.933	3046.333
EAA	2126.533	2670.8	2404.467	2159.267	1309.433
NEAA	2514.367	2478.333	2108.1	2471.667	1736.9

Means with the same superscript are not significantly different at experimental periods (P>0.05).
TFAA : total free amino acid; nd: not detected

Table 3. The fatty acid contents in fertilized eggs and yolk sac larvae of gilthead sea bream at different periods of development (% of total fatty acids; Mean±SD; n=3)

		prelarval stages after hatching(hours)			
Fatty acids	Egg	0^{th}	24^{th}	48^{th}	96^{th}
C6:0	0.0285±0.0021	nd	nd	nd	nd
C8:0	0.0125±0.0007	nd	nd	nd	nd
C10:0	0.013±0.0014	nd	nd	nd	nd
C11:0	nd	nd	nd	nd	nd
C12:0	0.16±0.0113	0.029±0.0014	0.029±0.000	0.036±0.000	0.032±0.000
C13:0	0.0275±0.0021	0.0185±0.0007	0.018±0.000	0.018±0.000	0.015±0.000
C14:0	3.1805±0.1774	3.584±0.1074	3.5±0.0155	3.3815±0.036	2.975±0.0226
C14:1	0.067±0.0014	0.0735±0.0049	0.0705±0.0035	0.0765±0.0063	0.0715±0.0007
C15:0	0.498±0.0141	0.5745±0.012	0.555±0.0028	0.5275±0.0049	0.4625±0.0049
C15:1	0.018±0.007	0.022±0.0014	0.017±0.0014	0.045±0.000	0.0275±0.0134
C16:0	18.066±0.5975	16.971±0.4574	17.291±0.0169	13.8225±0.0784	12.789±0.0311
C16:1	5.888±0.2107	7.9775±0.2877	7.696±0.0339	8.267±0.0834	7.3945±0.0417
C17:0	0.606±0.0056	0.663±0.0268	0.683±0.0042	0.6155±0.0007	0.5535±0.0049
C17:1	0.034±0.0014	0.0415±0.0021	0.0395±0.0007	0.0405±0.0021	0.04±0.0014
C18:0	3.157±0.0113	2.183±0.0028	2.4335±0.0148	1.745±0.0127	1.818±0.007
C18:1n9t	nd	0.1835±0.0021	0.1425±0.0021	0.175±0.0014	0.057±0.0509
C18:1n9c	20.671±0.0487	19.493±0.1541	20.167±0.0636	21.907±0.2107	25.215±0.0007
C18:2n6t	0.0225±0.0021	0.0185±0.0007	0.0195±0.0007	0.0215±0.0035	0.02±0.0028
C18:3n6	0.078±0.000	0.065±0.0028	0.0655±0.0007	0.03655±0.0049	0.061±0.0028
C18:2n6c	8.8945±0.0417	7.9655±0.0162	8.672±0.0763	9.459±0.0791	11.988±0.0226
C20:0	0.101±0.0084	0.068±0.0169	0.065±0.0028	0.057±0.0127	0.069±0.0056
C18:3n3	1.6525±0.0063	1.373±0.0551	1.553±0.048	1.6±0.0042	1.973±0.0028
C20:1n9	0.856±0.0226	0.391±0.0113	0.521±0.106	0.7255±0.0275	0.7935±0.0077
C21:0	0.6465±0.0106	0.518±0.0169	0.495±0.000	0.51±0.0268	0.4595±0.0106
C20:2	0.16±0.0042	0.1425±0.0445	0.103±0.0014	0.135±0.024	0.155±0.007
C20:3n3	0.4065±0.0162	0.26±0.0395	0.218±0.0014	0.3115±0.0431	0.3525±0.0091
C22:0	0.0415±0.0091	0.0895±0.0431	0.116±0.000	0.084±0.0593	0.019±0.0028
C22:2	0.0235±0.0049	0.036±0.000	nd	0.034±0.000	0.02±0.0042
C20:4n6	0.815±0.024	1.1985±0.0275	1.1115±0.0049	1.2615±0.0176	1.2835±0.0007
C22:1n9	0.068±0.0028	0.043±0.0141	0.033±0.000	0.0385±0.0106	0.0455±0.0035
C23:0	0.0435±0.0007	0.0235±0.0007	0.036±0.0127	0.104±0.0806	0.055±0.0268
C20:5n3	2.3255±0.0417	4.0935±0.0134	3.822±0.0141	3.781±0.0084	3.426±0.0226
C24:0	0.28±0.000	nd	nd	nd	0.143±0.0707
C24:1n9	0.406±0.065	0.0875±0.0021	0.331±0.0155	0.179±0.0777	0.054±0.0014
C22:6n3	14.761±0.5105	18.293±0.473	16.338±0.0346	15.9585±0.0134	14.608±0.1513
TFA	84.009±0.3698	86.481±0.3323	86.142±0.1852	84.9825±0.345	86.997±0.1972
MUFA	28.008±0.1619	28.312±0.4221	29.017±0.1463	31.454±0.3118	33.699±0.0084
PUFA	29.139±0.5642	33.446±0.6986	31.903±0.0735	32.627±0.0388	33.887±0.1668
SATURATED	26.862±0.7721	24.722±0.6088	25.221±0.0346	20.901±0.0721	19.411±0.0219
DHA/EPA	6.3474±0.1056	4.4689±0.1008	4.2748±0.0067	4.2207±0.013	4.2638±0.016
n-3/n-6	1.951±0.062	2.597±0.049	2.222±0.016	2.003±0.013	1.524±0.01
UD	15.99±0.3698	13.519±0.3323	13.858±0.1852	15.017±0.345	13.002±0.1972

TFA: total fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; UD: undefined; nd: not detected

14.761±0.5105, 0.815±0.024, 28.008±0.1619 and 29.139±0.5642, respectively. The most abundant FA at the start of exogenous feeding was 18:1n9c (25.215±0.0007), followed by 22:6n-3 (14.608±0.1513) and 16:0 (12.789±0.0311). The ratios of (n-3)/(n-6) PUFA in the fertilized eggs and at the beginning of exogenous feeding were 1.95 and 1.52, respectively.

Discussion

Early life history of marine fish larvae is complex phenomenon of growth and differentiations. Understanding the evolution of the biochemical compositions as well as the ontogeny of the digestive functions in the fertilized eggs and yolk sac stages of the marine fish larvae provides valuable source to better understand the nutritional requirements at the beginning of exogenous feeding.

Since all the physiological parameters could be varied during ontogenesis, the activity of only the cytosolic enzyme (LEU-ALA) is not enough to account for the evolution of the digestive maturation of enterocyte. The importance of the peptide digestion in the brush border membranes (BB) compared to that in the intracellular compartment could be expressed

by the ratio of the activities of BB peptidases (LAP) and cytosolic peptidase (LEU-ALA). It is known that the enzymatic activity ratio of LAP to LEU-ALA (LAP/LEU-ALA) is a good indicator of the digestive capacity (Cahu and Zambonino Infante, 1995). As commonly known, the digestive capacities of seabream larvae at the beginning of the exogenous feeding tended to be higher than fertilized eggs.

In present study, AP activities increased until the end of the experimental period. It is well known that AP is stimulated by phosphorylated substrates such as phosphoproteins and phospholipids (Shirazi *et al*., 1978; McCarty *et al*., 1980). The eggs and yolk sac larvae are excellent sources of phosphorylated substrates (Hertrampf, 1992). The results above are supported that acid and alkaline phosphatase activities were detected in the yolk of *Sparus aurata* by Sarasquete *et al*. (1993).

It is well known that the amylase activity is stimulated by dietary change. In other words, the dietary starch content can modulate the change in the amylase activity (Peres *et al*., 1998; Sheele, 1993). In present study, the dietary components were not used. Therefore, results showed that the variations in the amylase activities were not due to the dietary components but genetically programming before the exogenous feeding.

According to the results of the present study, tryptic activity tended to increase at the start of the exogenous feeding. The observed increase in tryptic activity in yolk sac larvae just prior to first feeding is also reported in recent literature (Alliot *et al*., 1977; Pedersen and Hjelmeland, 1988; Ueberschar *et al*., 1992; Chen *et al*., 2006). It can be assumed that this initial increase is a general feature of fish larvae that hatch at embryonic stages with large yolk sac.

Significant differences in free amino acid (FAA) concentrations of fertilized eggs and yolk sac larvae were observed through the experimental period. The predominant AAs observed in *S. aurata* eggs were the leucine, lysine, valine, alanine, proline, aspartic acid, phenylalanine and glutamic acid. This profile is consistent with a survey across a range of pelagic fish eggs in which leucine, lysine, valine, isoleucine alanine and serine were found predominant. This similarity between species is attributed to the hydrolysis of a common protein at the time of oocyte hydration (Ronnestad and Fyhn, 1993). EAA contents except for phenylalanine and threonine decreased significantly from fertilized egg to the start of exogenous feeding ($P<0.05$). In present study, the total decrease in EAA (38.42%) up to the first feeding stage was greater than that of NEAA (30.92%). In most fish species, yolk material provides the substrates for energy and growth during the egg and yolk sac stage. The general model assumed that yolk lipid is the main energy substrate (Fyhn, 1993; Polat *et al*., 1995; Sargent *et al*., 1995; Verreth *et al*., 1995; Naz, 2008) whereas yolk protein provides amino acids for tissue synthesis (Heming and Buddington, 1988). In marine fish eggs energy is partly derived from the FAAs pools (Fyhn, 1993; Ronnestad *et al*., 1992) while phospholipids fulfill other essential functions for growth and survival (Kanazawa et al., 1985; Sargent *et al*., 1993). The general consensus in present study is that FAA has a major role as an energy source.

In our study, MUFAs and PUFAs ratios in fertilized eggs of *S. aurata* were higher than SFA ratios. Results obtained in the study were supported by Bulut (2004). In addition, fertilized eggs and yolk sac larvae of *S. aurata* are rich in n-3 FAs, EPA (20:5n-3) and DHA (22:6n-3). It is well documented that the principal FAs found in the eggs of marine fish larvae were 22:6n-3, 20:5n-3, 16:0 and 18:1n9 (Mourente and Odriozola, 1990). On the other hand, the ratios of (n-3)/(n-6) PUFA in the fertilized eggs and the beginning of exogenous feeding were 1.951 and 1.524, respectively. This ratio is very low compared to similar ratios in many other species, both marine and freshwater (Naz, 2008; Sargent, 1995; Henderson and Tocher, 1987). Considering the relative high proportion of 22:6n-3 in the (n-3) PUFA, these low (n-3)/(n-6) ratios should be attributed to the elevated levels of 18:2n-6. Although marine fish generally contained lower (n-6) PUFA levels than freshwater fish, the results found in current study may also reflect the fatty acid composition of the broodstock diets, which may have contained elevated levels of linoleic acid from vegetable oils and of DHA from fish oils.

Our results revealed that the seabream eggs and yolk sac larvae contain a significant pool of FAAs and FAs. In addition, the changes in enzymatic activity observed from fertilized egg up to the first feeding have provided important information about digestive enzyme potentials of post larvae at the beginning of exogenous feeding. This information is of a great importance for the development of artificial feeds for marine fish larvae.

On the basis of the above-mentioned data, the nutrient dynamics in egg and yolk sac stages could be provided a model to overcome some problems observed in the larval stage of marine fish. Also, obtained results could be contributed concerning the nutritional requirements of seabream larvae at the onset of exogenous feeding. On the other hand, the fertilized eggs and yolk sac larvae contain the different molecular forms of the proteins except FAA. Thus, future efforts on the determination of changes in protein forms will contribute to formulize the most appropriate microdiets acceptable by larvae. In other words, further studies are needed to explain the absorption dynamics and role of peptides in marine fish larval nutrition.

References

Alliot, E., Pastoreaud, A. and Trellu, J. 1977. Evolution of enzymatic activities in the digestive system during the sea bass (*Dicentrarchus labrax*) larval life. Variations of proteinograms and zymograms. In: Proc. 3rd

Meeting ICES Working Group on Mariculture Brest, France, May 1977. Brest: CNEXO. France: 85-91.

AOAC, 1995. Hidrolysis Method. Phenomex EZ Faast GC-FID Hydrolized Aminoacid Analysis Kit.

Bessey, O.A., Lowry, O.H. and Brock, M.J. 1946. Rapid coloric method for determination of alkaline phosphatase in five cubic millimetres of serum. J. Biol. Chem., 164: 321–329.

Bradford, M.M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem., 72: 248–254.

Bulut, M. 2004. Biochemical composition of sea bass (*Dicentrarchus labrax* L., 1758) and sea bream (*Sparus aurata* L., 1758) eggs. E.U. Journal of Fisheries and Aquatic Sciences, 21(1-2): 129-132.

Chen, B.N., Oin, J.G., Kumar, M.S., Hutchinson, W. and Clarke, S.M. 2006. Ontogenetic development of digestive enzymes in yellowtail kingfish *Seriola lalandi* larvae. Aquaculture, 260(1-4): 264-271.

Cahu, C.L. and Zambonino Infante, J.L. 1995. Effect of the molecular form of dietary nitrogen supply in sea bass larvae: Response of pancreatic enzymes and intestinal peptidases. Fish Physiol. Biochem., 14: 209-214.

Cahu, C.L. and Zambonino Infante, J.L. 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture, 200: 161-180.

Finn, R.N., Ronnestad, I. and Fyhn, H.J. 1995. Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) Comp. Biochem. Physiol., 111(A): 647-671.

Fyhn, H.J. 1989. First feeding of marine fish larvae: are free amino acids the source of energy? Aquaculture, 80: 111-120.

Fyhn, H.J. 1993. Multiple functions of free amino acids during embryogenesis in marine fishes. In: B.T. Walther and H.J. Fyhn (Eds.), Physiological and biochemical aspects of fish development. University of Bergen, Bergen, Norway: 299-308.

Garces, R. and Mancha, M. 1993. One-step lipid extraction and fatty acid methyl esters preperation from fresh plant tissues. Analytical Biochemistry, 211: 139-143.

Heming, T.A. and Buddinton, R.K. 1988. Yolk absorption in embryonic and larval fishes. Fish Physiology, 11(A): 408-446.

Henderson, R.J. and Tocher, D.R. 1987. The lipid composition and biochemistry of freshwater fish. Prog Lipid Res., 26: 281-347.

Hertrampf, W.J. 1992. Feeding aquatic animals with phospholipids II. Fishes Lucas Meyer Publication, 11: 70 pp.

Kanazawa, A., Teshima, S. and Sakamoto, M. 1985. Effects of dietary lipids, fatty acids, and phospholipids on growth and survival of prawn (*Penaeus japonicus*) larvae. Aquaculture, 50: 39-49.

Kolkovski, S., Tandler, A., Kissil, W.G. and Gertlez, A. 1993. The effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata,* L.) larvae. Fish Physol. and Biochem., 12(3): 203-209.

Maroux, S., Louvard, D. and Baratti, J. 1973. The aminopeptidase from hog intestinal brush border. Biochim. Biophys Acta., 321: 282-295.

Mccarty, D.M., Micholson, J.A. and Kim, Y.S. 1980. Intestinal enzyme adaptation to normal diets of different composition. Am. J. Physiol., 239: 445-451.

Metais, P. and Bieth, J. 1968. Determination de l'a-amylase par une microtechnique. Ann. Biol. Clin., 26:133–142.

Mourente, G. and Odriozola, J.M. 1990. Effect of brodstock diets on lipid classes and their fatty acid composition of larvae of gilthead sea bream (*Sparus aurata*). Fish Physiol. and Biochem., 8(2): 103-110.

Naz, M., 2008. Ontogeny of biochemical phases of fertilized eggs yolk sac and larvae of Sea bass (*Dicentrarchus labrax* L., 1758). The Israeli Journal of Aquaculture-Bamidgeh, 60(2): 113-120.

Nicholson, J.A. and Kim, Y.S. 1975. A one-step L-amino acid oxidase assay for intestinal peptide hydrolase activity. Anal Biochem., 63: 110–117.

Parra, G. and Yufera, M. 2000. Feeding, physiology and growth responsein first-feeding gilthead seabream *(Sparus aurata* L.) larvae in relation toprey density. J. Exp. Mar. Biol. Ecol., 243: 1–15.

Pedersen, B.H. and Hijelmeland, K. 1988. Fate of trypsin and assimilation efficiency in larval herring (*Clupea harengus)* following digestion of copepods. Mar Biol., 97: 467-476.

Peres, A., Zambonino Infante, J.L. and Cahu, C.L. 1998. Dietary regulation of activities and mRNA levels of trypsin and amylase in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem., 19: 145-152.

Polat, A., Conceicao, L., Sarıhan, E. and Verreth, J. 1995. The protein, lipid and energy metabolism in eleuthero-embryos and starving larvae of the Afican catfish *Clarieas gariepinus* (Burchell). ICES Mar Symp. Series, 201: 74-79.

Ronnestad, I., Finn, R.N., Groot, E.P. and Fyhn, H.J. 1992. Utilization of free amino acids related to energy metabolism of developing eggs and larvae of lemon sole *Microstomus kitt* reared in the laboratory. Mar. Ecol. Prog. Ser., 88: 195-205.

Ronnestad, I. and Fyhn, H.J. 1993. Metabolic aspects of free amino acids in developing marine fish eggs and larvae. Rev. Fish. Sci., 1: 239-259.

Ronnestad, I., Kowen, W.M., Tandler, A., Harel, M. and Fyhn, H.J. 1994. Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). Mar. Biol., 120: 187-196.

Ronnestad, I., Thorsen, A. and Finn, R.N. 1999. Fish larval nutrition: a review of recent advences in the roles of amino acids. Aquaculture, 177: 201-216.

Sarasquete, M.C., Polo, A. and Gonzales de Canales, M.L. 1993. A histochemical and immunohistochemical study of digestive enzymes and hormones during the larval development of the sea bream, *Sparus aurata*. *L.* Histochem. J., 25(6): 430-437.

Sargent, J., Henderson, R.J. and Tocher, D.R. 1989. The lipids. In: J.E. Halver (Ed.) Fish Nutrition, 2nd edition Academic Press Inc., London: 153-217.

Sargent, J., Bell, J.G., Bell, M.W., Henderson, R.J. and Tocher, D.R. 1993. The metabolism of phospholipids and polyunsaturated fatty acids in fish. Aquac. Fundament. Appl. Res., 43: 103-124.

Sargent, J.R., 1995. Origins and functions of lipids in fish eggs: nutritional implicitiations. In: N.R. Bromage and R.J. Roberts (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Scientific Publications, Oxford: 353-372.

Seoka, M., Takii, K., Takaoka, O., Nakamura, M. and Kumai, H. 1997. Biochemical phases in embryonic red sea bream development. Fish Sci., 63: 122-127.

Sheele, G.A. 1993. Regulation of pancreatic gene expression in response to hormones and nutritional substrates. In: V.L.W. Go, J.D. Gardner, F.P. Brooks,

E. Lebenthal, E.P. G.A. DiMagno Sheele (Eds.), The Pancreas: Biology,Pathobiology and Disease 2nd Edn. Raven Press, New york: 103-120.

Shirazi, S.P., Colston, K.W. and Butterworth, P.J. 1978. Alkaline phosphatase: a possible transport for inorganic phosphate. Biochem. Soc. Trans., 6: 933-935.

Sivaloganathan, B., Walford, J., Ip, Y.K. and Lam, T.J. 1998. Free amino acids and energy metabolism in eggs and larvae of sea bass, *Lates calcarifer*. Mar. Biol., 131: 695-702.

SPSS, 1993. SPSS for Windows Base System User's Guide, release 8.0.2. Chicago, USA.

Tseng, H.C., Grendell, J.H. and Rothman, S.S. 1982. Food, duodenal extracts, and enzyme secretion by the pancreas. Am. J. Physiol., 243: 304–312.

Ueberschar, B., Pedersen, B.H. and Hjelmeland, K. 1992. Quantification of trypsin with radioimmunoassay in herring larvae (*Clupea harengus*) compared with a highly sensitive fluorescence technique to determine tryptic enzyme activity. Mar Biol., 113: 469-473.

Verreth, J., Polat, A., van Herwaarden, H., Conceicao, L. and Huisman, E. 1995. A comparison of methods to study energy resource partitioning in early life stages of freshwater fish, with special reference to the African catfish *Clarias gariepinus* (Burchell). ICES Mar Symp Series, 201: 57-63.

Yufera, M., Polo, A. and Pascual, E. 1993. Changes in chemical composition and biomass during the transition from endogenous to exogenous feeding of *Sparus aurata* L. (Pisces, Sparidae) larvae reared in the laboratory. J. Exp. Mar. Biol. Ecol., 167:149–161

Yufera, M., Fernandez-Diaz, C. and Pascual, E. 1995. Feeding rates of gilthead seabream, *Sparus aurata* L., larvae on microcapsules. Aquaculture, 134: 257-268.

Yufera, M., Sarasquete, M.C. and Fernandez-Diaz, C. 1996. Testing protein-walled microcapsules for the rearing of first-feeding gilthead seabream (*Sparus aurata* L.) larvae. Mar. Freshwater Res., 47: 211-216.

Yufera, M., Pascual, E. and Fernandez-Diaz, C. 1999. A highly efficient microencapsulated food for rearing early larvae of marine fish. Aquaculture., 177: 249-256.

Zambonino Infante, J.L. and Cahu, C.L. 2001. Ontogeny of the gastrointestinal tract of marine fish larvae. Comp. Biochem. and Physiol., 130(C): 477-487.